JOURNAL OF BACTERIOLOGY, June 1998, p. 3174–3180 Vol. 180, No. 12
0021-9193/98/$04.00+0

Multiple Transcriptional Control of the *Lactococcus lactis trp* Operon

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Received 2 December 1997/Accepted 23 March 1998

The *Lactococcus lactis trpEGDCFBA* operon is preceded by a noncoding leader region. Transcriptional studies of the *trp* operon revealed three transcripts with respective sizes of 8 kb (encompassing the entire operon), 290 bases, and 160 bases (corresponding to parts of the leader region). These transcripts most likely result from initiation at the unique P_{trp} promoter, transcription termination at either T1 (upstream of the *trp* operon) or T2 (downstream of the *trp* operon), and/or processing. Three parameters were shown to differentially affect the amount of these transcripts: (i) following tryptophan depletion, the amount of the 8-kb transcript increases 300- to 500-fold; (ii) depletion in any amino acid increased transcription initiation about fourfold; and (iii) upon entry into stationary phase the amount of the 8-kb transcript decreases abruptly. The tryptophan-dependent transcription control is exerted through transcription antitermination.

Tryptophan is an amino acid whose synthesis is one of the most energy requiring (29), and thus any repression of unnecessary synthesis would be advantageous to the cell. Conversely, a sufficient tryptophan supply is critical to protein synthesis. It is therefore to be expected that tryptophan biosynthesis is tightly controlled in the cell. This makes the tryptophan biosynthetic pathway an attractive model for the study of gene regulation. The *trp* genes and the regulation of their expression in many prokaryotes have been described. These studies have revealed a striking contrast between a high conservation of the tryptophan biosynthetic enzymes and a great diversity of the regulatory mechanisms. This diversity is believed to reflect the adaptation of the microorganisms to their particular way of life (9).

In most bacteria, expression of the *trp* genes is coordinately controlled by tryptophan. In *Escherichia coli*, this control is exerted through repression of transcription initiation, as well as through transcription attenuation (for a review, see reference 34). This latter mode of control involves stalling of the ribosome at tryptophan codons during translation of a leader peptide coding region, which leads to the formation of an antiterminator structure. This mechanism is also thought to operate in *E. coli* relatives (33), in *Brevibacterium lactofermentum* (27), and in *Rhizobium meliloti* (1). In *Bacillus subtilis* and its relative *Bacillus pumilus*, termination is controlled by the tryptophan-dependent binding of TRAP protein, which prevents the formation of an antiterminator structure (for a review, see reference 15). In fluorescent pseudomonads, the *trp* genes are found at different locations on the chromosome and their expression is not coordinately controlled by their end product, tryptophan. In these organisms, the transcription of *trpB* and *trpA* is activated by the TrpI regulatory protein in the presence of indole 3-glycerophosphate, the substrate of TrpBA (5).

In *Lactococcus lactis*, a gram-positive bacterium with a low G+C content, the *trp* operon has also been characterized (2). It contains all seven structural genes necessary for tryptophan biosynthesis in the order *trpEGDCFBA* and is preceded by a leader region containing a putative transcription terminator. This organization is evocative of a coordinated gene regulation involving transcription antitermination (2). The *trp* leader also exhibits primary sequence and predicted secondary structure conservation with the "T-box" family of leader regions upstream of many aminoacyl-tRNA synthetase genes and some amino acid biosynthesis operons in a number of gram-positive bacteria (17). Some of these genetic systems have been shown to be regulated by an antitermination mechanism controlled by interaction with the cognate uncharged tRNA (16). The strong conservation of the leader regions of all these systems, including the lactococcal *trp* operon, has led to the suggestion that they share a common regulatory mechanism (18).

We describe here the transcription pattern of the *trp* operon of *L. lactis*. We identified three parameters controlling transcription. (i) Tryptophan depletion is followed by a 300- to 500-fold increase in the amount of the *trp* transcript. This control is mediated by transcription antitermination. (ii) Depletion in any amino acid increases transcription initiation about fourfold. (iii) The amount of the *trp* transcript decreases abruptly upon entry of the cells into stationary phase.

MATERIALS AND METHODS

Bacterial strains and growth conditions. *L. lactis* subsp. *lactis* IL1403 (6) and derivatives were grown as described previously (2). The chemically defined medium (CDM) for *L. lactis* is adapted from previously described media (23, 25, 28) and contained (per liter) sodium acetate, 1 g; ammonium citrate, 0.6 g; KH_2PO_4, 9.0 g; K_2HPO_4, 7.5 g; $MgCl_2$, 0.2 g; $FeCl_2$, 5 mg; $CaCl_2$, 50 mg; $ZnSO_4$, 5 mg; $CoCl_2$, 2.5 mg; alanine, 0.24 g; arginine, 0.12 g; asparagine, 0.34 g; cysteine, 0.17 g; glutamine, 0.51 g; glycine, 0.17 g; histidine, 0.11 g; isoleucine, 0.20 g; leucine, 0.47 g; lysine, 0.35 g; methionine, 0.12 g; phenylalanine, 0.28 g; proline, 0.68 g; serine, 0.34 g; threonine, 0.23 g; tryptophan, 0.10 g; tyrosine, 0.29 g; valine, 0.33 g; *para*-aminobenzoic acid, 10 mg; biotin, 10 mg; folic acid, 1 mg; nicotinic acid, 1 mg; pantothenic acid, 1 mg; riboflavin, 1 mg; thiamine, 1 mg; pyridoxine, 2 mg; cyanocobalamin, 1 mg; orotic acid, 5 mg; 2-deoxythymidine, 5 mg; inosine, 5 mg; DL-6,8-thioctic acid, 2.5 mg; pyridoxamine, 5 mg; adenine, 10 mg; guanine, 10 mg; uracil, 10 mg; xanthine, 10 mg; and glucose, 2.5 g. *E. coli* TG1 (13) was grown as described previously (2).

DNA manipulations. Plasmid DNA was extracted as previously described (2). *E. coli* cells were transformed according to the standard procedure with $CaCl_2$

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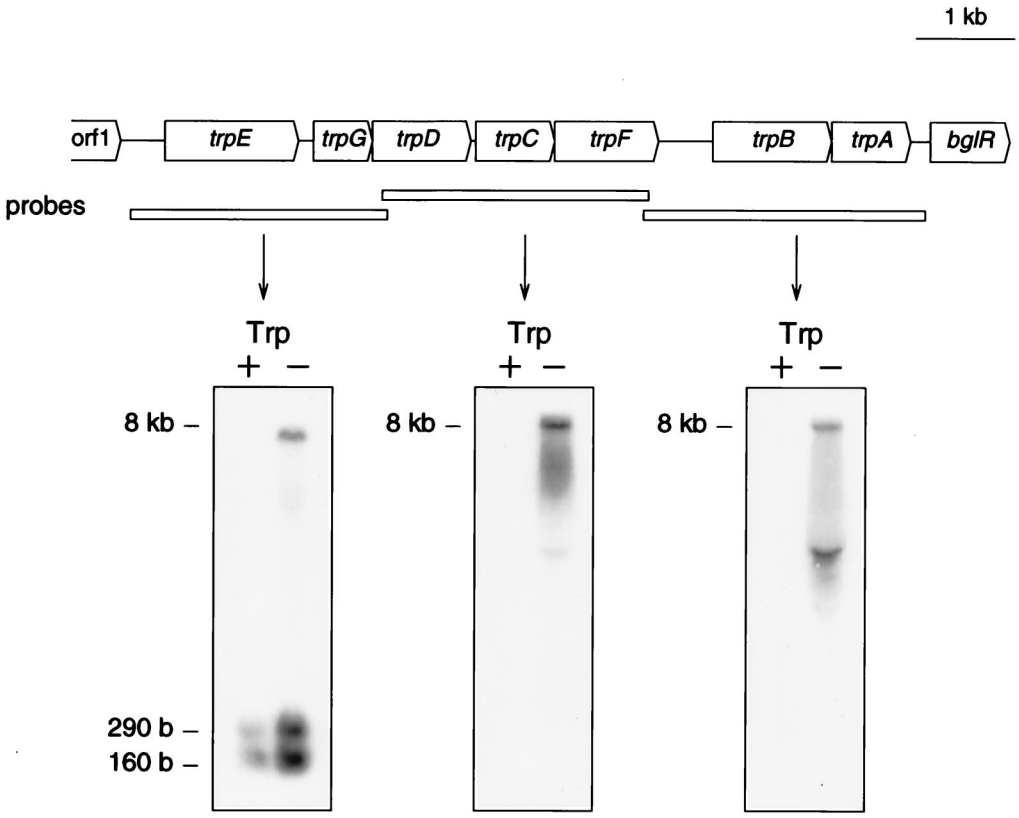

FIG. 1. Identification of the *trp* transcripts. The upper part of the figure presents the genetic structure of the *trpEGDCFBA* operon (2, 3). The three open boxes represent DNA fragments used as probes in Northern blot experiments; they were synthesized by PCR and correspond to the sequence coordinates 592 to 3210, 3193 to 5866, and 5846 to 8632 in sequence M87483, respectively (2). Northern hybridization was carried out on RNA prepared from cells noninduced (+) or induced (−) with tryptophan (Trp) as described in Materials and Methods.

(26). *L. lactis* was transformed by an electroporation technique (20). Other molecular techniques were carried out by established procedures (26).

Extraction and analysis of RNA. Total RNA was extracted from *L. lactis* by an adaptation of the method of Glatron and Rapoport (14). Cells from 25-ml cultures were sedimented by centrifugation, and the cell pellet was resuspended in 500 μl of cold TE (10 mM Tris, 1 mM EDTA; pH 8.0). The cell suspension was added to a 2-ml screw-cap microcentrifuge tube containing 0.6 g of glass beads (0.1-mm diameter), 170 μl of 2% Macaloid slurry (26), 500 μl of water-saturated phenol-chloroform (1:1), and 25 μl of 20% sodium dodecyl sulfate. Cells were disrupted by shaking in a Mini-Beadbeater-8TM Cell Disrupter (Biospec Products, Barttlesville, Okla.) for 5 min. After centrifugation at 15,000 rpm for 15 min, the aqueous supernatant, which contained the RNA, was extracted with 1 volume of phenol-chloroform, precipitated with ethanol, resuspended in TE, and stored at −80°C. For Northern blot analysis, 20 μg of total cellular RNA was denaturated by treatment with glyoxal, separated by electrophoresis through a 1% agarose gel, and transferred by capillary blotting to a nylon membrane (Hybond-N; Amersham). Alternatively, RNA was separated by electrophoresis through a 6% polyacrylamide gel and transferred by electroblotting to a nylon membrane. The 0.16- to 1.77-kb and 0.24- to 9.5-kb RNA ladders from Gibco-BRL were used as molecular size markers. The membranes were stained for rRNA and RNA markers with methylene blue (32). Hybridization used either DNA fragments radiolabeled by nick translation or synthetic oligonucleotides labeled at their 5′ termini by transfer of γ-^{32}P with T4 polynucleotide kinase. Hybridization and washing of the membranes were conducted under standard conditions. Quantification of the amounts of probe hybridized was done with a PhosphorImager (Molecular Dynamics, Sunnyvale, Calif.).

Oligonucleotides. Oligonucleotides 1 (complementary to nucleotide coordinates 585 to 607 in sequence GenBank M87483 [2]), 2 (complementary to nucleotides 617 to 638), 3 (complementary to nucleotides 697 to 716), 4 (complementary to nucleotides 721 to 744), 5 (complementary to nucleotides 773 to 794), 6 (complementary to nucleotides 823 to 840), and 7 (complementary to nucleotides 8511 to 8529) were synthesized with a Beckman Oligo-1000 DNA synthesizer according to the instructions accompanying the apparatus.

Primer extension analysis. Oligonucleotide primers were 5′ end labeled with [γ-^{32}P]ATP by using T4 polynucleotide kinase and used in primer extension reactions run with Avian reverse transcriptase (Gibco BRL). Briefly, 10 μg of total RNA and 5 pmol of labeled oligonucleotide were hybridized following heating at 85°C for 10 min and cooling down for ca. 30 min to 42°C. The hybridized primer was then extended with 5 U of Avian reverse transcriptase for 1 h at 42°C in the conditions recommended by the supplier. The reaction product was precipitated with ethanol, resuspended in TE buffer with 50% formamide, and electrophoresed on DNA sequencing gels alongside DNA sequencing reactions with the same primer.

Plasmid constructions. Plasmids were constructed by standard methods (26). When needed, the ends of the restriction fragments were made blunt by treatment with T4 DNA polymerase before joining by treatment with T4 DNA ligase. Recombinant plasmids were first selected in *E. coli* cells before transfer into *L. lactis* by electroporation. The plasmids (see Fig. 4) are derivatives of pGKV210, a promoter-screening vector containing the promoterless *cat-86* chloramphenicol resistance determinant (30) or pGKV259, which is pGKV210 in which the strong lactococcal P_{59} promoter has been cloned upstream of *cat-86* (31). pIL1801 was obtained by cloning the *Sty*I-*Hin*dIII fragment from the *trp* leader (coordinates 451 to 886) between the *Sac*I and *Sal*I sites of pGKV210. pIL1804 was obtained by cloning the *Xmn*I-*Hin*dIII fragment (coordinates 579 to 886) in the *Sal*I site of pGKV259. pIL1805 was obtained by deletion of a *Dra*III-*Cla*I fragment from pIL1804. pIL1807 was obtained by deletion of a *Sam*I-*Nru*I fragment from pIL1804.

RESULTS

Identification of the *trp* transcripts. The *trp* transcripts were analyzed in cells incubated in the presence or absence of tryptophan. *L. lactis* cells were grown in CDM containing tryptophan to mid-exponential-growth phase (optical density at 600 nm of between 0.5 and 0.6), centrifuged, and resuspended in CDM containing either 100 μg of tryptophan per ml (noninducing conditions) or no tryptophan (inducing conditions) for 30 min. Total RNA was extracted, and *trp* transcripts were

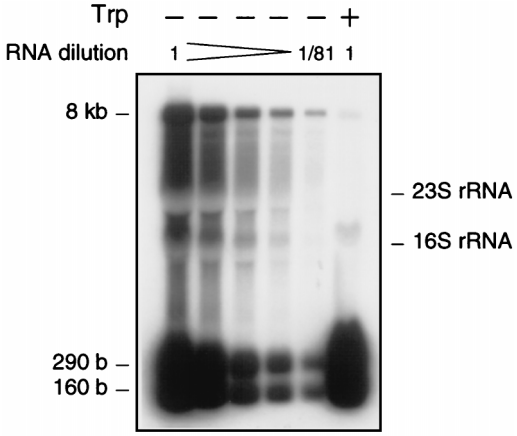

FIG. 2. Modulation of the amount of the *trp* transcripts in response to tryptophan availability. The amounts of the different transcripts in cells incubated for 30 min with (+) or without (−) tryptophan were compared in a Northern blot experiment. Oligonucleotide 6 was used as a probe.

analyzed by Northern blot hybridization. Three overlapping DNA fragments encompassing the entire leader region and the operon were used as probes (Fig. 1). All three probes revealed an 8-kb transcript in induced cells. The probe encompassing the leader region of the *trp* operon also revealed two small transcripts. Their size was determined after electrophoresis in 6% polyacrylamide gel and Northern blotting to be 290 and 160 bases (b), respectively (data not shown). They were three to four times more abundant in induced than in noninduced cells. This effect, however, was not tryptophan dependent since a similar increase was also observed when any single amino acid was omitted from the CDM (data not shown).

Prolonged exposure of Northern blots revealed that the 8-kb transcript was 300- to 500-fold less abundant in noninduced than in induced cells (Fig. 2). This also revealed additional bands within the smear of incomplete or degraded 8-kb transcript. Some bands corresponded to the electrophoresis artifacts due to the presence of the 23S and 16S rRNA, a finding common in Northern blot experiments (19, 21, 22). Some other faint bands may represent discrete breakdown products. However, their low abundance relative to that of the 8-kb transcript, indicates that this polycistronic mRNA was not subjected to a significant processing.

The seven *trp* genes therefore form an operon, whose transcription is tightly controlled by tryptophan. Transcription of the two small transcripts is slightly modulated by amino acid availability.

Mapping of the transcripts and identification of regulation signals. The three *trp* transcripts were mapped more precisely by using restriction fragments or synthetic oligonucleotides as probes. The results, which are summarized in Fig. 3, revealed that the two ends of the 8-kb transcript were located close to the putative transcription promoter P_{trp} and the transcription terminator T2, respectively. Both small transcripts had their 3′ end close to transcription terminator T1. The 5′ ends of the transcripts were determined by priming total RNA isolated from noninduced or induced cells with the appropriate oligonucleotide. The 290- and 160-b transcripts had their 5′ ends at positions 551 and 681 to 683, respectively (Fig. 4), suggesting that their 3′ ends will be close to position 840 and corresponding to transcription arrest at T1. Attempts to define the 5′ end of the 8-kb transcript with an oligonucleotide complementary only to this transcript were unsuccessful, most probably because of a premature arrest of the reverse transcriptase at the T1 terminator secondary structure. Hybridization of the 8-kb transcript with oligonucleotide probe 1 or 2 suggests that its 5′ end is at sequence position 551. However, it is still possible that a fraction of the 8-kb molecules have a 5′ end corresponding to sequence position 681 to 683.

These results suggest that the 8-kb and the 290-b transcripts are most likely initiated at the putative consensus P_{trp} promoter lying at position 515 to 543. To test this hypothesis, the *Sty*I-*Xmn*I DNA fragment (position 451 to 581) was inserted upstream of a promoterless *cat-86* gene between the *Sac*I and *Sma*I sites of pGKV210 (30). The resulting plasmid (pIL1802) conferred resistance to 8 μg of chloramphenicol per ml on *L. lactis* IL1403, whereas the same strain containing the vector plasmid only was sensitive to 2 μg of chloramphenicol per ml (data not shown), indicating that the P_{trp} promoter is functional.

The 160-b transcript may originate either from a nonconsensus lactococcal promoter localized downstream of P_{trp} or from transcript processing. To distinguish these two possibilities, different segments of the *trp* leader region were cloned into plasmid pGKV210 (30) (Fig. 5). Plasmid pIL1801, carrying P_{trp} and the T1 terminator (*Sty*I-*Hin*dIII region; position 451 to 886), produced both the 290- and the 160-b transcripts. Deletion of the P_{trp}-containing *Sty*I-*Xmn*I region (position 451 to 581) yielded pIL1807, which no longer produced these transcripts. To exclude possible deletion or inactivation of a nonconsensus promoter in pIL1807, the leader segment lacking P_{trp} was cloned downstream of the lactococcal P_{59} promoter on a pGKV210 derivative (31). This resulted in plasmid pIL1804, which produced both a 440- and a 160-b transcript. The 440-b transcript has the size expected for a transcript initiated at P_{59} and terminated at the T1 transcription terminator. The 160-b transcript has the size expected for a processing product of the 440-b one. These results demonstrate that the 160-b transcript is a processing product.

Both the 290- and the 160-b transcripts have their 3′ ends close to sequence position 840, which corresponds to the putative transcription terminator T1 (2). To demonstrate that this region has a termination function, we compared transcription

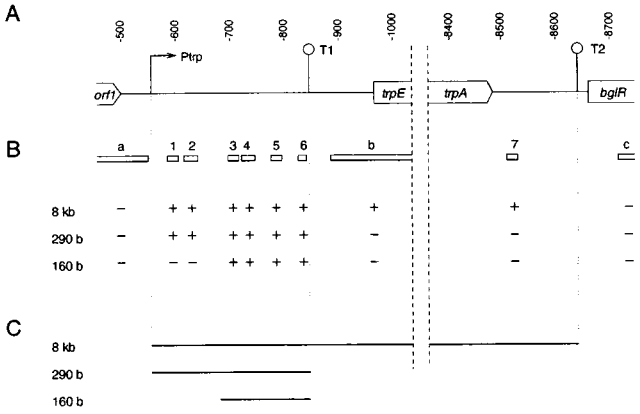

FIG. 3. Structure and Northern blot analysis of the *trp* operon. (A) Structure of the leader and 3′ region of the *trp* operon. Numbers refer to nucleotides in sequence M87483 (2). Boxes correspond to genes. T1 and T2 indicate transcription terminators, and P_{trp} indicates the transcription promoter. (B) Northern analysis of the *trp* transcripts. Total RNA from induced cells was hybridized with either restriction fragments a to c or oligonucleotides 1 to 7 (see Materials and Methods). Hybridization (+) and absence of signal (−) are indicated. (C) Mapping of the *trp* transcripts. This transcription map combines results from Northern analysis and 5′ end mapping of the transcripts.

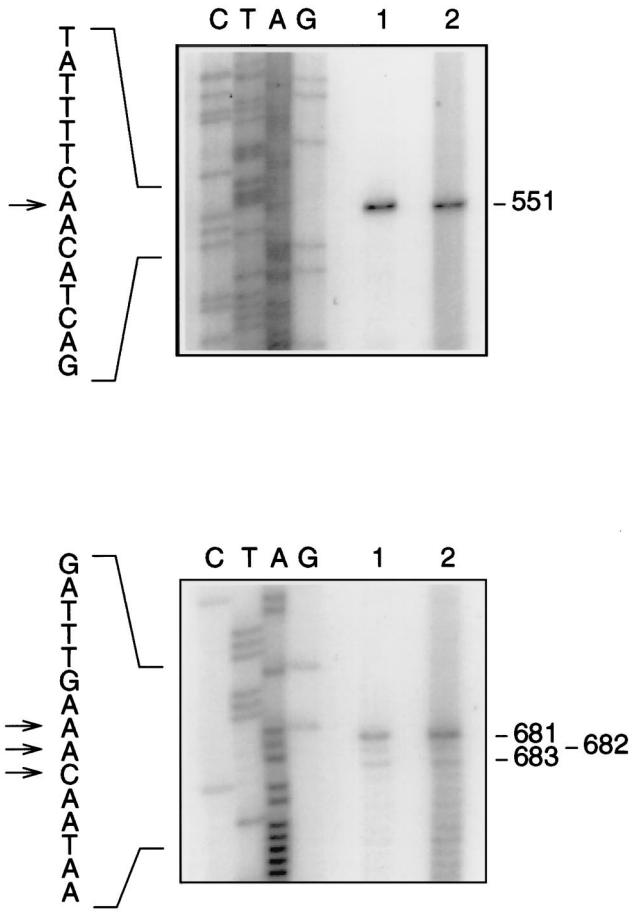

FIG. 4. Determination of the 5′ ends of the *trp* transcripts by primer extension. RNA isolated from noninduced cells (lanes 1) or induced cells (lanes 2) was used in a primer extension assay with oligonucleotide 5 as described in Materials and Methods. The extension products were run alongside sequencing reaction products obtained with the same primer. Only regions of the gel containing bands of extension product are shown. The localization of the 5′ ends within the sequence is indicated by arrows.

from plasmids pIL1804 and pIL1805, which only differ by the presence of the *Dra*III-*Hin*dIII leader region carrying T1 (position 799 to 886), between P_{59} and *cat-86* (Fig. 5B). Cells containing pIL1804 produced the expected 440-b transcript and its 160-b processing product and were sensitive to 4 μg of chloramphenicol per ml. By contrast, cells containing pIL1805 produced a 1.3-kb transcript and were resistant to 14 μg of chloramphenicol per ml. These results demonstrate that the *Dra*III-*Hin*dIII region contains a transcription terminator.

Taken together, our results suggest that all transcripts are initiated at P_{trp}. The 8-kb transcript terminates at T2, and the two small transcripts terminate at T1. The 160-b transcript is produced by processing.

***trp* transcription is controlled by antitermination.** The 300- to 500-fold increase in the amount of the 8-kb transcript induced by tryptophan depletion could result either from an antitermination mechanism acting on T1 or from a controlled decay of the 8-kb transcript. To distinguish these two hypotheses, we measured the stability of the 8-kb transcript in the presence or absence of tryptophan. Decay of the 8-kb transcript was measured following addition of rifampin either in the presence or in the absence of tryptophan (Fig. 6). The observed kinetics of decay were similar in both conditions with half-lives in the range 5 to 7 min, indicating that modulation of the amount of the 8-kb transcript was not mediated by a change in its stability but rather by a tryptophan-controlled antitermination mechanism.

Amount of *trp* transcripts is controlled by growth phase. The results presented thus far were obtained in cells in mid-exponential-growth phase shifted to either the presence or the absence of tryptophan for 30 min. Amounts of *trp* transcripts were also examined during steady-state growth. Omission of tryptophan from the medium did not affect the growth rate of IL1403 and only resulted in a 30-min-longer lag phase (Fig. 7A). The amounts of 290- and 160-b transcripts in cells grown in either the presence or the absence of tryptophan remained relatively constant and parallel during the period of growth examined until the entry into the stationary phase, when an abrupt drop in the amount of the 290-b transcript occurred accompanied by a simultaneous increase in the amount of the 160-b transcript (Fig. 7B and C). The 8-kb transcript was only detectable in cells grown in the absence of tryptophan, where it was ca. 30-fold less abundant than the small transcripts on a molar basis. The amount of the 8-kb transcript also suddenly decreased upon entry into stationary phase (Fig. 7C). This indicates the existence of an additional control of the amount of the *trp* transcript that responds to the growth phase.

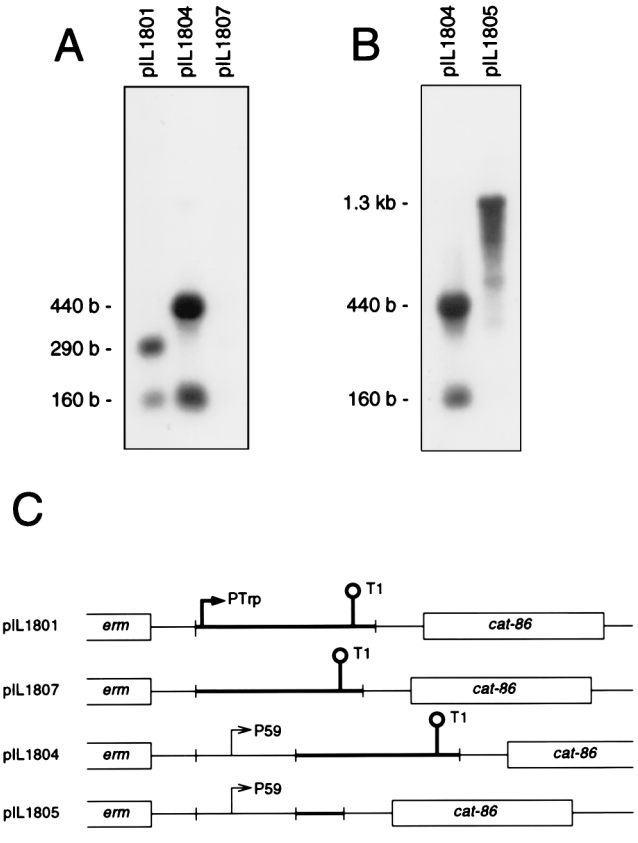

FIG. 5. Transcript production by different plasmids. (A and B) RNA extracted from *L. lactis* cells containing the indicated plasmids was hybridized in a Northern blot experiment with appropriate probes. (C) Schematic representation of the relevant regions of the plasmids used. Segments of *trp* leader are indicated by heavy lines. Since the plasmids used have high copy numbers, transcripts originating from plasmids outnumber transcripts originating from chromosome, which are therefore not visible in these experiments.

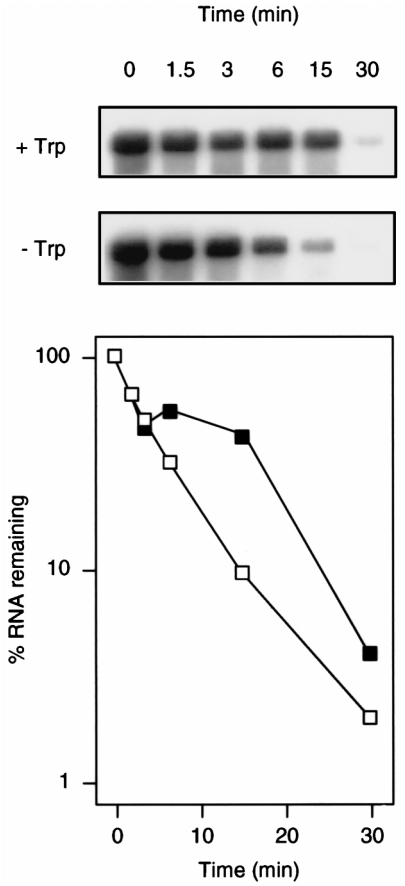

FIG. 6. Effect of tryptophan on the half-life of the 8-kb transcript. Production of the 8-kb transcript was induced by resuspending IL1403 cells for 30 min in CDM without tryptophan. Total RNA was isolated at time intervals after addition of 120 μg of rifampin per ml and either 100 μg of tryptophan per ml (+Trp) or no tryptophan (−Trp) and analyzed in Northern blot experiments with oligonucleotide 6 as the probe. Decay kinetics were measured in cells suspended in the presence (■) or absence (□) of tryptophan.

DISCUSSION

Characterization of the *trp* transcripts and transcriptional signals. Transcription of the lactococcal *trp* operon gives rise to three transcripts. An 8-kb mRNA encompasses the entire *trp* operon, and two 290- and 160-b transcripts correspond to early terminated transcripts from the *trp* leader region. The functionality of the putative transcription promoter P_{trp} was demonstrated. No evidence for the existence of other promoters within the operon was obtained. All *trp* transcripts are thus likely to be initiated at P_{trp}. The *trp* operon is flanked by two putative transcription terminators, T1 and T2. Evidence was presented here that the DNA region containing T1 has a transcription terminator function. Recently, characterization of regulatory mutants by Frenkiel et al. (12) presented definitive evidence that this function was due to T1. T2 is most probably functional, since it is likely to be involved in the transcriptional control of the downstream gene *bglR* by β-glucoside sugars (3, 4).

Processing of the transcripts. The 5′ end of the 160-b transcript was shown to be produced by cleavage of a larger transcript. A 160-b transcript was also produced from the *trp* leader carried on a plasmid in *B. subtilis* or *E. coli* (29a), suggesting the involvement of an endonuclease conserved within bacteria. Processing of *B. subtilis* T-box leader transcripts has been reported previously in six of nine systems examined. The processing sites were located close upstream of the transcription terminator (7). In the case of the *thrS* leader, processing was shown to be due to a homolog of RNase E (8) and to participate in the regulation by increasing the stability of the processed *thrS* mRNA following threonine depletion (7). The processing observed in *L. lactis* most probably corresponds to a different phenomenon since the cleavage site is located at a different relative position and the cleavage efficiency was not affected by tryptophan availability. Our results, however, do not exclude the possibility that a second cleavage site, located close upstream of the transcription terminator, also exists in the lactococcal *trp* leader.

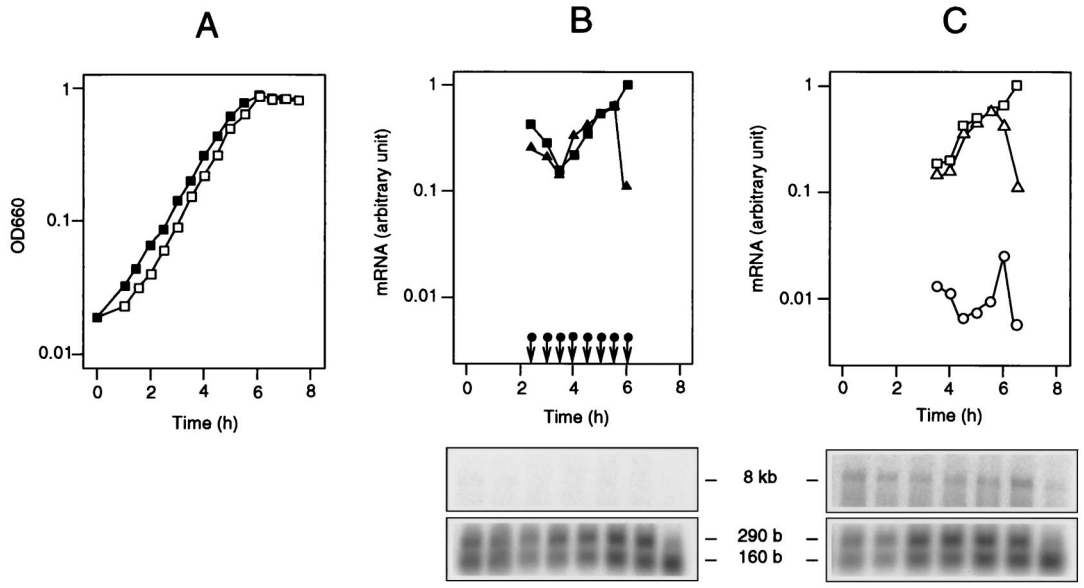

FIG. 7. Fate of *trp* transcripts during growth cycle. (A) Growth curve of IL1403 in CDM with tryptophan (■) or without tryptophan (□). (B) Fate of *trp* transcripts in cells grown in CDM with tryptophan. (C) Fate of *trp* transcripts in cells grown in CDM without tryptophan. Symbols: ■ and □, 160-b transcript; ▲ and △, 290-b transcript; ● and ○, 8-kb transcript. Downward arrows indicate that the amount of RNA is below the detection limit.

Origin of the transcripts. Our data suggest that transcription is initiated at the unique P_{trp} promoter and that most transcripts are terminated early, at transcription terminator T1, leaving the 290-b transcript. Some transcripts may read through T1 and are extended through the entire operon, up to transcription terminator T2, giving rise to the 8-kb transcript. Cleavage of a fraction of the transcripts by an endoribonuclease will generate the 160-b transcript (and possibly a shortened 8-kb transcript). The fact that the RNA 5′ fragment resulting from the cleavage was not detected in our Northern blotting experiments is most readily explained by the action of 3′-to-5′ exoribonucleases, which rapidly degrade 3′ unprotected transcripts in bacteria (10, 11).

Transcription controls. Three parameters were shown to influence the amount of *trp* transcripts: (i) tryptophan depletion increases 300- to 500-fold the amount of the 8-kb transcript; (ii) depletion in any amino acid increases the amount of the 290- and 160-b transcripts about fourfold; and (iii) upon entry into stationary phase, the amount of the 8-kb and 290-b transcripts decreases abruptly by about fivefold.

The amount of 8-kb transcript is strongly modulated by tryptophan availability. This is exerted by antitermination at terminator T1 and represents the major transcription control. This confirms earlier speculations on *trp* regulation in *L. lactis* that were based on the presence of the putative terminator T1 upstream of the operon (2) and on sequence and secondary structure similarities between the *trp* leader and other gram-positive genes or operons known to be controlled by transcription antitermination (17).

The stringency of this tryptophan-dependent control compares with those observed in *E. coli* or *B. subtilis*, where tryptophan biosynthesis was repressed 500- and 400-fold, respectively, in the presence of tryptophan (15, 34). This suggests that a tight control of this biosynthetic pathway is necessary, possibly to avoid an energy waste to the cell.

Transfer of the cells to a medium lacking tryptophan or any other amino acid produced a fourfold increase in the amount of the 290- and 160-b transcripts. The observation that the stability of these transcripts is not affected by tryptophan availability (data not shown) indicates the existence of a control of transcription initiation at P_{trp} which represents a second minor level of regulation.

A third level of control of the *trp* operon was observed upon entry of the cells into stationary phase, when an abrupt decrease in the amount of the 8-kb and the 290-b transcripts was observed. This was accompanied by a simultaneous increase in the amount of the 160-b transcript. This observation could be explained by an increased activity of the endoribonuclease which produces the 160-b transcript during exponential growth. Control of tryptophan biosynthesis by growth phase makes perfect sense in view of the *L. lactis* physiology. In this organism, entry into stationary phase is accompanied by a dramatic energy shortage (24). This mechanism could therefore be advantageous to the cell in suppressing tryptophan biosynthesis in conditions of energy limitation.

ACKNOWLEDGMENTS

We gratefully acknowledge Costa Anagnostopoulos and Marie-Christine Chopin for their kind help in the preparation of the manuscript.

This work was supported by Bridge-Biot-CT91-0263 contract of the Commission of the European Communities.

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